

The University of Chicago

THE EFFECT OF CALCIUM
DEFICIENCY ON RESPIRATION OF
ETIOLATED PEA, CORN, AND
SQUASH SEEDLINGS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1938

By
WENDELL R. MULLISON

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EFFECT OF CALCIUM DEFICIENCY ON RESPIRATION OF ETIOLATED SEEDLINGS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 500

WENDELL R. MULLISON

Introduction

Few references in the literature are concerned with the effect of nutrient deficiency on respiration. SPOEHR and MCGEE (10) worked on the effect of amino acids supplied in nutrient solution to excised *Helianthus* leaves. HAMNER (2) studied the effect of nitrogen supply on the respiration rate of tomato and wheat. GREGORY and SEN (1) reported the effect of nitrogen and potassium on the respiration of excised barley leaves. JONES (3) worked on the respiration of etiolated wheat seedlings as affected by phosphorus. The present work deals with the respiration rate of tops and roots of etiolated plants in relation to calcium supply. Carbohydrate, protein, and fatty seeds were employed in this study.

Material and methods

Pea seeds of the Prince Edward variety, single cross hybrid selected corn obtained from Funk Brothers Seed Company, Bloomington, Illinois, and Hubbard squash seeds were used. The weight of each seed varied at most not more than 15 mg. from that of the other seeds employed of the same kind. All seeds were sterilized in sodium hypochlorite, then soaked in distilled water for several hours until definite signs of swelling appeared. At that time they were placed in groups of ten in special beakers filled with sterile quartz sand previously thoroughly flushed with distilled water. Eight beakers were used for each determination. They were placed for germination in the darkroom in which the runs were made. Each day the plants of four beakers were supplied with a complete nutrient solution (7) and the other four with a minus calcium nutrient solution. This latter was the same as the complete nutrient solution except that calcium nitrate was replaced by potassium nitrate. Determinations of the respiratory rate were made when the seedlings were about a week old.

The temperature was kept as close as possible to 29°C ., with a variation of only 1° . The darkroom was lighted by means of a 7 watt red bulb for only the brief time necessary each day to water the plants and to place them in readiness for the respiration determinations.

The apparatus which was built for this work was a modification of the one described in detail by MITCHELL (5), and was based upon the same principles. The amount of CO_2 given off by the plants was determined by the conductivity method. A CO_2 -free stream of air was passed through a closed chamber in which the plants were growing. From here it was bubbled through humidifiers and then drawn through absorption towers. These towers contained 50 cc. of $\frac{1}{10}$ normal lithium hydroxide solution to which had been added 1 per cent butylic alcohol to lower the surface tension. The air flow was regulated to move at the rate of 20 liters per hour through the absorbent, the gases being dispersed as fine bubbles not more than 2 mm. in diameter. The towers were immersed in a water bath kept at a constant temperature that varied not more than 0.01°C . After passage through the absorption towers, the remaining gases flowed through copper tubing into a manifold where the negative pressure was controlled by a valve and indicated by a manometer. From the manifold, a tube led directly to an air pump which created the slight negative pressure required to draw air through the entire system. Sixteen sets of this apparatus were assembled as one unit and operated by a single air pump, accommodating the tops and roots of the eight sets of plants.

The towers were standardized separately by titration with $\frac{1}{10}$ NHCl , using phenolphthalein as the indicator. Thereafter the amount of CO_2 absorbed in the lithium hydroxide solution was determined by using a wheatstone bridge to measure the increased resistance of the solution.

Each chamber in which the determinations were made consisted of two parts, the lower part being the 1.5 liter beaker in which the plants were grown. It was provided with two outlets, one at the center of the bottom and the other one-fourth of the distance from the top. The upper part of the chamber was placed over the tops of the plants at the time of each run. It consisted of 4 inch pyrex tubing sealed at one end and with two side tubes for the incoming and

outgoing streams of air. In order to determine the respiratory rate of the roots as contrasted with that of the tops, they were separated from each other by a seal made of a mixture of paraffin, vaseline, and mineral oil that had a melting point of 40° C. The top part of the chamber was sealed to this paraffin mixture by a paste of clay and castor oil. The two compartments of the chamber were tested by means of a manometer to insure that they were air tight. Before the run the chambers were flushed with CO₂-free air for an hour. Each run involved approximately eighty plants and was of four hours' duration.

Investigation

EXPERIMENTS WITH PEAS

Calcium deficiency became apparent immediately upon germination. The hypocotyls of the minus calcium seeds grew more slowly than those given the plus calcium solution, so that by the end of the

TABLE 1
FOUR-HOUR DETERMINATION ON 7-DAY-OLD PEAS

CALCIUM	PART OF PLANT	CO ₂ RESPIRED PER 80 PLANTS (MG.)	CO ₂ RESPIRED PER SINGLE PLANT (MG.)
Plus	Tops.....	60	3.5
	Roots.....	216	
Minus	Tops.....	32	2.4
	Roots.....	196	

fourth day the radicles of the latter were twice the length of the former. In the minus calcium plants at this time a cessation of growth of the primary root occurred, after which a few laterals grew feebly for a short time. Some of the minus calcium seeds, although producing roots, failed to push their epicotyls above the sand level. The calcium deficiency soon became so severe that necrosis appeared at the tips of the plants. Table 1 gives the data of a representative run.

EXPERIMENTS WITH CORN

Corn was also markedly sensitive to calcium deficiency. Here again the rate of growth of the minus calcium plants was slower than

that of the plus calcium. For the most part in the calcium deficient plants the leaves did not break through the coleoptile, while the plus calcium of the same age had two leaves unfurled. The gross appearance of the corn roots was not noticeably affected by the lack of calcium. Data are shown in table 2.

TABLE 2
FOUR-HOUR DETERMINATION ON 6-DAY-OLD CORN

CALCIUM	PART OF PLANT	CO ₂ RESPIRED PER 80 PLANTS (MG.)	CO ₂ RESPIRED PER SINGLE PLANT (MG.)
Plus	Tops.....	148.8	6.96
	Roots.....	328.0	
Minus	Tops.....	79.2	5.27
	Roots.....	342.4	

EXPERIMENTS WITH SQUASH

Of the three plants used, squash was the slowest to show the effect of calcium deficiency. The rate of growth was retarded by lack of calcium, but necrosis did not set in at once. The length of the hypocotyl was affected while the respiratory rate was but little changed. Table 3 gives the results obtained.

TABLE 3
FOUR-HOUR DETERMINATION ON 6-DAY-OLD SQUASH

CALCIUM	PART OF PLANT	CO ₂ RESPIRED PER 80 PLANTS (MG.)	CO ₂ RESPIRED PER SINGLE PLANT (MG.)
Plus	Tops.....	200	4.4
	Roots.....	152	
Minus	Tops.....	200	4.3
	Roots.....	146	

Discussion

The investigations which have been recorded regarding the function of calcium in plants indicate that it is concerned with permeability, toxic relationships, the actual formation of new cells, and the synthesis of protoplasm. TRUE (11) reported that calcium made

other nutritive elements physiologically available, this effect on permeability apparently being governed by calcium soaps and proteinates. Not only does calcium make other ions available, but in its complete absence plants undergo a leaching of material from their roots. Calcium also aids in preventing toxicity of other elements which in its absence prove harmful (8). NIGHTINGALE has described calcium deficiency symptoms in detail, noting that it first appears in meristematic regions.

With this understanding of the necessity of calcium to the plant, it follows that respiration in its absence would be modified. These results correspond with those obtained by other workers on the effects of nutrient deficiencies upon respiration. HAMNER (2) found that the respiration of tomato and wheat plants decreased when the nitrogen supply was limited. GREGORY and SEN (1) found that the effect of nitrogen and potassium deficiencies was to retard respiration. The latter work was done on excised barley leaves, and not on the entire plant; however, great care was taken to select only leaves of comparable maturity. They emphasize the fact that as individual leaves mature, their respiratory rate changes. JONES reported that the respiration rate of wheat seedlings increased upon the addition of phosphorus.

HAMNER first reported that the respiration rate of the roots and tops of any given plant or group of plants may be very different. Too often in considering respiration that of the tops alone is taken into account, thus neglecting root respiration which in tomato plants supplied with full nutrient solution and wheat seedlings is much more than that of the tops. In the work reported in the present paper it was found that the respiration rate of the roots in etiolated pea and corn seedlings was more than twice that of the tops. This is owing to the fact that there are many actively growing regions in fibrous root systems as contrasted with relatively few meristematic regions in the stem. Where there is less metabolic activity due to the lessened number of actively growing cells, one would expect a slower rate of respiration. In the squash seedlings this great difference in the rate of respiration of the roots as contrasted with that of the tops was not found. The respiration of the roots and tops was about the same, with the tops respiring slightly more. This was probably due to the fact that the cotyledons in

squash come above ground and are the source of food for the seedlings until the leaves are formed. Even so the respiration of the tops was not a great deal more than that of the roots. In corn and pea the cotyledons were not raised above ground, and therefore their respiration was included with that of the roots.

Of the three types of seeds (carbohydrate, protein, and fatty) of the same age, the seedlings which were of the high carbohydrate type respired most, the protein type respired least, and the fatty type was intermediate. Fatty seeds may absorb large quantities of oxygen in the formation of carbohydrates from fats during germination.

Although when roots and tops were separated, the roots of the minus calcium corn plants respired more than those of the plus calcium, the total respiration of the minus calcium plants was always less than that of the plus calcium. At the present time this increased respiration of the minus calcium corn roots presents an interesting unsolved problem. In this connection it may be noted that corn is a member of the grass family, which has been reported to require relatively little calcium (9). GREGORY and SEN reported a slightly increased rate of respiration due to a moderate potassium starvation, and only when the starvation became acute did the rate fall. If the roots do not suffer from calcium deficiency and are normal, however, their respiratory rate should be the same as the controls, whereas actually it exceeded them. In addition it is difficult to explain the severe minus calcium symptoms shown by the tops.

The effect of lack of calcium on squash is the least marked of the three types. Although minus calcium plants were much smaller in size, their respiration was approximately the same as those supplied with complete nutrients. There were few signs of necrosis in the minus calcium plants. Even though the plus calcium ones were larger, their respiration was but slightly more. It may be that the minus calcium squash plants had not yet begun to show a severe calcium deficiency, there having been enough available calcium stored in the seed to serve for immediate needs. This stored supply of available calcium probably varies with the type of seeds used, which may account for the difference in the response of the three kinds used in this work.

These results of calcium deficiency on respiration may be interpreted by considering lack of calcium a limiting factor in anabolic

plant processes; hence with fewer cells and less protoplasm, a lowered respiration rate must occur.¹

A fact worthy of note was the effect on growth of the seedlings immediately after germination when supplied with the minus calcium nutrient solution. This caused a decided decrease in rate of development that was most pronounced in the case of pea. Pea seeds grew and formed radicles approximately 2 inches long, and small epicotyls, after which growth stopped and was not appreciably resumed. Whether this is due to the toxic action of other elements in the absence of calcium, which seems most likely, or to the effect on permeability or its lack in anabolism is not definitely known. Pea seeds contain calcium, but perhaps not much is available for germination. MAQUENNE and DEMOUSSY (4) reported finding a stimulating effect of calcium on pea seed germination. NIGHTINGALE (6) pointed out the extreme immobility of bound calcium in tomato plants. He also stressed the fact that when new cells are formed, calcium must be present, not only for the development of the middle lamella, but also for the synthesis of protoplasm. Since in seeds the amount available for use is small, much of the calcium necessary for the developing of new tissue from nearly the beginning must come from outside the seed. This factor, of course, would probably vary greatly with different types of plants.

Summary

1. The effect of calcium deficiency upon the rate of respiration of etiolated seedlings was studied. Seeds used in this study were pea, corn, and squash, examples respectively of the three general classes of seeds: protein, carbohydrate, and fat. The plants were divided into two parts, the tops and the roots, and the respiration of each was studied separately.

2. Total respiration under conditions of plus and minus calcium nutrition was highest in the corn seedlings, least in the pea seedlings, and intermediate in the squash.

3. Total respiration of the minus calcium plants was in every case less than that of the plus calcium plants.

¹ Work on calcium nutrition of the bean plant (GAUCH, H. G., Responses of the bean plant to calcium deficiency. In Press.), done subsequent to this investigation, shows that with plants given a minus calcium nutrient solution, the toxic effects are due to the other ions in the absence of calcium.

4. Lack of calcium was most noticeable in the greatly lowered respiration of the minus calcium tops as compared with that of the plus calcium tops.

5. Calcium deficiency lowered the respiration of the roots of pea and squash, but increased the root respiration of corn.

6. Roots of pea and corn respired more than twice as much as did their tops.

7. Respiration of the roots and tops of squash was about equal, although the tops respired more than the roots.

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